

Methanococcus medium—general recipe (McC or McCas or nitrogen-free (N-free) medium)

Have tubes and/or bottles and stoppers in anaerobic chamber at least an hour ahead of time. For plates, have petri dishes in chamber at least the day before.

	<u>100 ml:</u>	<u>200 ml:</u>	<u>500 ml:</u>	<u>10 liters:</u>
<u>Combine before boiling:</u>				
H ₂ O	50 ml	100 ml	250 ml	
General Salts solution	50 ml	100 ml	250 ml	
OR N-free General Salts solution				
NaHCO ₃				
for liquid (hi pressure):	0.5 g	1 g	2.5 g	
for agar (low pressure):	0.2 g	0.4 g	1 g	20 g
NaCl	2.2 g	4.4 g	11 g	220 g
K ₂ HPO ₄ soln	1 ml	2 ml	5 ml	100 ml
FeSO ₄ soln	0.5 ml	1 ml	2.5 ml	50 ml
Trace Minerals (1,000X)	0.1 ml	0.2 ml	0.5 ml	10 ml
Vitamin soln (100X)	1 ml	2 ml	5 ml	100 ml
Resazurin soln	0.1 ml	0.2 ml	0.5 ml	10 ml
NaAcetate·3H ₂ O	0.14 g	0.28 g	0.7 g	14 g
Difco yeast extract (McC)	0.2 g	0.4 g	1 g	20 g

OR casamino acids (McCas)

OR neither (N-free)

Difco Noble Agar (agar medium)	1.5 g	3 g	7.5 g	
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(For agar medium and N-free medium: Add just before boiling:

dithiothreitol	0.05 g	0.1 g	0.25 g)	
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Boil under stream of N₂:CO₂ **OR** (agar medium) autoclave.

Add immediately after boiling or autoclaving while under stream of N₂:CO₂ (**omit for N-free medium**):

cysteine·H ₂ O·HCl	0.05 g	0.1 g	0.25 g	5 g
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Allow to cool under stream of N₂:CO₂. Precipitate (liquid medium) should go back into solution.

Dispense 5 ml per tube in anaerobic chamber. Swirl to keep any precipitate evenly suspended while dispensing. **OR** (agar medium) cool under a stream of N₂:CO₂ and pour plates.

Tubes: Exchange gas to 80:20 H₂:CO₂ (McC or McCas) or N₂CO₂ (N-free).

Tubes: Autoclave

Tubes: before inoculation, add sulfide (anaerobic sterile solution), 0.1 ml per 5-ml tube. Adjust gas composition and pressure as desired.